Early Journal Content on JSTOR, Free to Anyone in the World

This article is one of nearly 500,000 scholarly works digitized and made freely available to everyone in the world by JSTOR.

Known as the Early Journal Content, this set of works include research articles, news, letters, and other writings published in more than 200 of the oldest leading academic journals. The works date from the mid-seventeenth to the early twentieth centuries.

We encourage people to read and share the Early Journal Content openly and to tell others that this resource exists. People may post this content online or redistribute in any way for non-commercial purposes.

Read more about Early Journal Content at http://about.jstor.org/participate-jstor/individuals/early-journal-content.

JSTOR is a digital library of academic journals, books, and primary source objects. JSTOR helps people discover, use, and build upon a wide range of content through a powerful research and teaching platform, and preserves this content for future generations. JSTOR is part of ITHAKA, a not-for-profit organization that also includes Ithaka S+R and Portico. For more information about JSTOR, please contact support@jstor.org.

STUDIES IN PNEUMONIA AND PNEUMOCOCCUS INFECTIONS.*

Edward C. Rosenow.†

(From the Pathological Laboratory of Rush Medical College.)

CONTENTS.

Introduction.
- I. Pneumococcemia in Pneumonia.
  - Review of the literature.
  - Material and technic.
  - Results of blood cultures, of smears.
  - Diagnostic value of blood cultures.
  - Prognostic value of blood cultures.
- II. Pneumococcemia in Relation to the Localization of Lobar Pneumonia.
- III. Pneumococcemia in Relation to the Leucocytosis and Crisis of Pneumonia.
  - Leucocytosis in Pneumococcus Infection of the Rabbit.
- IV. Action of Blood and Serum on Pneumococci.
- V. Agglutination of the Pneumococcus.
- VI. The Production of Acid by the Growth of Pneumococci in Pneumonic Serum.
- VII. Hemolysis in Fatal Pneumococcus Infections in Rabbits.
- VIII. The Differentiation of Pneumococci and Streptococci.
- IX. Conclusions.

Bibliography.

INTRODUCTION.

In this work, which was undertaken at the suggestion of Dr. Hektoen, an attempt has been made to establish more accurately (1) the frequency and time of pneumococcus invasion of the blood in pneumonia, and its relation to the leucocytosis, and particularly to the crisis, as well as the diagnostic and prognostic value of blood cultures in this disease; (2) to study the aggluti-

---

* Received for publication February 1, 1904.

† This work has been carried out under the Alumni Fellowship of Rush Medical College. Preliminary reports upon some parts of the work have been published in *Trans. Chi. Path. Soc.*, 1903, 5, p. 265, and in *Medicine*, 1903, 9, p. 435.

nating, bactericidal, and other actions of pneumonic and other blood serum. In the course of the work attention became directed also to certain phases of the biology of the pneumococcus and of pneumococcus infection of the rabbit.

I. PNEUMOCOCCEMIA IN PNEUMONIA.

REVIEW OF THE LITERATURE.

The work of recent investigators shows remarkable differences in the frequency with which positive results have been obtained in blood cultures during life in lobar pneumonia.

The Klemperer brothers never found the pneumococcus in the blood, notwithstanding the fact that numerous cases were examined. Belfonti in many cases obtained only six positive results. Pässler found the Streptococcus longus and the bacillus of Friedländer once each in six fatal cases, while in thirty-eight cases that recovered the pneumococcus was found in the blood only once. Libman in 1894 demonstrated pneumococci in the blood during life in six of sixteen cases. In two of these they were found only in smears, the cultures remaining sterile. Three of the positive cases ended fatally, while only three of the twelve negative cases died. He used 5 c.c. of blood for agar plates. Kühnau, by the same method, but using twice the amount of blood, obtained only one positive result in nine cases. This case ended fatally. Two of the eight negative cases died. Kohn, also by the same method, grew pneumococci in nine out of a total of thirty-two cases. Seven of the positive cases died, while only two of the negative cases terminated fatally. Sereni in twenty-three cases found diplococci three times. Sello examined forty-eight cases, with twelve positive findings. Ten of these ended fatally; twenty-seven of the negative cases recovered. Pieraccini, in 1899, found the pneumococcus in the blood in eleven out of twenty-eight cases of pneumonia. In 1899 White made cultures from nineteen cases, ten of which were fatal. Diplococci were obtained in but three cases; all of these ended fatally. He used 5 c.c. of blood in agar and bouillon tubes. Banti in 1890 found pneumococci in cultures in eighteen of twenty-nine cases. Cole in 1902 reports nine positive findings in thirty cases. All of the positive and four of the negative cases ended fatally. The earliest time at which cultures were obtained was three days before death. In four negative fatal cases cultures were made a number of times. Bouillon and litmus milk were principally used. The author lays considerable stress upon the importance of diluting the blood well, to "overcome its bactericidal action," and believes that the finding of the organism in cultures of the blood is of bad omen. Nazari found cocci in the blood during life in four of seven cases. Baduel recovered the pneumococcus from the blood in fifty-five of fifty-seven cases, in some cases as late as twenty-five days after the crisis. In 1900 Silverstrini and Sertoli report fifteen positive results in sixteen cases. They think that the number of pneumococci frequently, but not always, bears a direct relation to the severity of the infection. Berghini found them in every one of eight cases, and believes them to be constant in the blood of pneumonics regardless of the gravity of the case. Casati as early as 1893 obtained positive results in twenty five consecutive cases by means of animal inoculations. Positive results were obtained as early as the second day. Fraenkel himself found the pneumococcus only in 20 per cent. of a large series examined, using the agar-plate method, but later he found them constantly by using bouillon as the culture medium. He shares the opinion of Prochaska that the organisms are present in every case, but that this does not necessarily mean a fatal prognosis. Prochaska in 1901 reports in two articles his results of blood cultures in fifty cases of pneumonia. In every case the diplococcus was found. Only one culture was made from each patient. He used bouillon and agar for his culture media, the former giving the more constant results. He lays no particular stress upon the degree of the dilution of the blood. Of the fifty cases, only twelve ended fatally. Positive results were obtained as early as the second day, and in one case as late as three days after the crisis. He believes his good results were due to the use of large quantities of blood (10 c.c.), in bouillon, and to great care in the examination of the cultures.

MATERIAL AND TECHNIC.

The cases examined were taken at random in the wards of the Cook County and Presbyterian Hospitals, and I am gratefully indebted to the attending physicians and the resident staffs for the interest taken and the many kindnesses shown.

After careful disinfection of the skin about the elbow by the use of green soap, 5 per cent. carbolic acid, and alcohol, the blood, 7 c.c. in the early and 10 to 13 c.c. in the later cases, was drawn from either the median basilic or cephalic vein. With great care to avoid contamination, so far as that is possible, 5–7 c.c. of blood was used for cultural purposes, while the rest was transferred to a 50 c.c. graduate, slanted until the blood coagulated, and then put into the ice-chest upright to permit the clear serum to drain off, which was then used for agglutination and other tests. In a small proportion of cases the white staphylococcus was found, presumably due to air infection, or perchance it was carried from the skin by the point of the needle. It is well to pass the end of the needle through a flame after withdrawing it so as to destroy all bacteria that may have been picked up from the skin, and that might otherwise be washed into the media with the blood while inoculating the flasks.

Bouillon (plain and glucose, 1 per cent.; acidity, 1 per cent.) and litmus milk mostly were used for the original cultures, the former giving the more constantly positive results, and of the two kinds of bouillon one proved about as good as the other. Glycerin bouillon (5 per cent.) was found to possess no special advantage over that of the others. In the early cases high dilutions were used (1:100 or 1:200), while later 1 part of blood to 50 or 75 parts of medium was employed. After incubation at 37.5° C. from eighteen hours to several days, smear preparations will show, usually, numerous diplococci. If the growth is at all abundant, a turbidity and brownish discoloration of the bouillon takes place, which is quite characteristic. The color reminds one of rusty sputum, and is due to the change in the hemoglobin of the blood.

Libman attributes this to the excessive acidity produced. The litmus milk is usually promptly acidified and coagulated. As pointed out by Cole, the capsule can usually be easily demonstrated in this medium. In all cases subcultures were made; the characteristic shape and staining reactions were noted in order to determine accurately the identity of the organism.

RESULTS.

Blood cultures.—In the total number of 145 cases studied, positive results were obtained in 132. In two cases (Nos. 35 and 24) the cultures remained sterile, but smear preparations directly from the blood showed the presence of undoubted pneumococci. A single blood culture was made in each of these cases on the eighth day of the disease. In the former of these there was a capillary leucocytosis of 42,000, while in the vein of the arm at the same time the blood showed a leucocytosis of only 30,000. In four of the negative cases (Nos. 10, 11, 15, and 38) cultures were made on the twelfth, thirteenth, fifteenth, and twenty-first days of the disease respectively, with normal temperatures in each instance. The blood was drawn mostly for other purposes and the cultures made incidentally. In two additional negative cases (Nos. 18 and 26) cultures were made on the second day of the disease. In case No. 104 the culture was made 36 hours after the crisis, with a negative result, and in No. 112 cultures were made on the day of the crisis, with a leucocyte count of 13,800. In only one of the negative cases (No. 55) is it difficult to explain the negative result. In this case three cultures were made, one on the day of crisis, one on the day following, and the third four days later, when there was an extension to the left lower lobe, after a previous massive consolidation of the entire right lung, in which resolution now appeared to be fairly well established. There was a leucocytosis of 30,000. Smears from the blood on the day of crisis, however, showed undoubted diplococci.

In most cases but one culture was made. However, in a number several cultures were made to determine any relationship that might exist between the crisis and pneumococcemia. The percentage of failures to obtain growths within thirty-six hours before crisis, and more especially on the day of crisis, seems to be higher than on the fourth or fifth day of the disease. Nor is the development, as a rule, as luxurious. In four instances organisms grew after what appeared to be in each instance a typical crisis, but in only one of these was the growth at all abundant. Cultures were made from four to thirty-six hours after crisis in eight cases.

Otherwise positive results were obtained in all stages of the disease; in one instance, where pneumonia developed in a patient in the ward, the organism was obtained in pure culture twelve hours after the initial chill, before there were any definite physical findings. This case ran a typical course, with more delirium than usual. The pneumococcus was demonstrated in the blood in four other cases before a positive diagnosis could be made. Cultures were made six times on the second day, with three positive and three negative results; on the third day four times, with three positive findings. The fourth, fifth, and six days gave the highest percentage of positive results, except the period within the last twenty-four hours before death in the fatal cases.

The organism in three cases, especially, showed rather marked tendencies toward chain formation. Two of these showed distinct capsules, and resembled in every other way typical pneumococci; while the other, growing in still larger chains, with no capsule, but resembling very closely indeed the pneumococcus in its cultural characteristics, except that it only acidified milk, might be considered a variety of streptococcus; but from the fact that the serum of this patient (who, as the other two, passed through a typical attack) agglutinated pneumococci (as did also the others) of different strains, one might be equally justified in considering it a variety of Fraenkel's diplococcus. The tendency of the pneumococcus to form chains in some instances, especially when cultivated in milk, was well brought out in a number of observations. While making the routine subcultures on the various media, and also when purposely studying this point, it was noticed that very early in milk (twelve hours after inoculation) undoubted pneumococci would sometimes form long chains which resembled the chains of Streptococcus longus in every way, whereas later the typical encapsulated lanceolate diplococcus predominated upon the same medium, as it did from the beginning upon the other media. I have found the use of blood agar a good method for differentiating pneumococci from streptococci (p. 308). It might be of interest to note that Dr. Weaver has found that pneumonic serum frequently possesses some, and at times very strong, agglutinating power over streptococci. Nine strains of streptococci were tested, obtained from various sources. Friedländer's bacillus was never observed in the blood cultures.

The mortality in this series of 40 per cent. shows that cases of rather marked severity have been studied, for the mortality of the total number of cases in the wards from which the cases were selected has been more nearly 35 per cent. Two cases of post-pneumonic gangrene showed pneumococci in the blood. The administration of antiseptics, such as creosote and guaiacol carbonate, do not seem to have any influence upon the presence of pneumococci in the blood.

Blood-smears.—By persistent search pneumococci were demonstrated in smears from the blood in forty-seven cases. In three of these the cultures remained sterile. Again, the organisms have been found repeatedly in the sera after being kept in the ice-chest for various lengths of time (in one instance as long as seventy days), and in three of these the original blood cultures also remained sterile. The positive results by this direct method of examination have been as numerous in the cases that recovered as in those that died. On the other hand, the negative findings are more numerous in the former. Various staining methods were tested, and it has been found that staining the film (after it has been fixed to the cover-glass in a corrosive sublimate-acetic acid solution) with Löffler's methylene blue is the most satisfactory. It may be contended that the diplococci found in the smears may have been contaminations; but the fact that chemically cleaned, sterilized cover-slips were used, carried to the bedside in sterile test-tubes, and replaced in the test-tube after the film had been made in a sterile manner, with forceps, then brought to the laboratory, placed in the fixing solution, and then treated with a carefully controlled staining solution, can leave little doubt that the diplococci found were present in the blood at the time it was drawn. The fact that the organisms were so numerous in some instances makes one raise the question whether or not there might not have been a multiplication of germs in the blood upon the cover-slips. This possibility was excluded by fixing one film immediately before it was dry, and placing the other in the incubator for twenty-four hours. In every instance where pneumococci were found in the film which had been incubated they were also found in the film which was fixed immediately, and there appeared to be an equal number in both. In only three instances did I find what appeared to be diplococci within polymorphonuclear leucocytes. Other observers have found pneumococci in smears, Orthenberger in six fatal cases. Libman found them in cover-slips in two cases where the cultures remained sterile.

Diagnostic value of blood cultures.—In a number of cases in this series blood cultures have been of positive value in the diagnosis. In one case of severe sepsis in which typhoid was considered in the diagnosis the blood culture showed it to be a case of pneumococcemia probably due to a local inflammatory process in the nasal cavity. In six cases influenza or broncho-pneumonia was diagnosed, while the blood cultures contained pneumococci. In five cases pneumococci were demonstrated in the blood before any physical signs were present.

Prognostic value of blood cultures.—Authors differ remarkably in regard to the prognostic value of the demonstration of pneumococci in the blood. Fraenkel, Prochaska, Baduel, Berghini, Casati, and other recent workers attribute only small or no prognostic significance to pneumococcemia in pneumonia, because present in nearly all cases. On the other hand, Krauss and Sello, White, Pane, Cole, Pieraccini, and others consider invasion of the blood by pneumococci a bad sign. Why this great difference of opinion? In most instances conclusions are drawn from insufficient data; again, others seem to conclude that because they fail to grow the organism its absence is established—a most misleading conclusion. I believe the differences in the results are explainable wholly on the score of recent improvements in technic, to wit: the use of large quantities of blood in liquid media, especially bouillon, and of high concentration of blood instead of high dilutions to overcome the so-called bactericidal action of the blood. All who have used the plate method have had positive results in only a small percentage of cases, while those who have used liquid media and animal inoculations have had almost uniformly positive results.

Eliminating the somewhat more uniformly positive results obtained during the last twenty-four hours of life, and the higher percentage of negative results upon the day of crisis in the cases with recovery, a careful analysis of the results earlier in the disease fails to show any difference in the number of times positive results were obtained in the fatal and non-fatal, and therefore less severe, cases. Hence we conclude with Fraenkel, Prochaska, Sittmann, and others that pneumococcemia in pneumonia is of no special prognostic value.

II. PNEUMOCOCCEMIA IN RELATION TO THE LOCALIZATION OF LOBAR PNEUMONIA.

That pneumonia may be the secondary localization of a primary blood invasion does not seem altogether improbable, because the clinical picture of many cases of lobar pneumonia reminds one strongly of rapidly fatal pneumococcemia with no special localization, and because in this series invasion of the blood stream seems to have been primary in point of time in five cases.

In the course of this work pneumonia resembling that in man resulted in two rabbits following intraperitoneal inoculation of pneumococci. The pneumococcus was isolated in pure culture from the blood in each instance. Washbourn has had similar results. Fraenkel, especially, mentions the development of pneumonia after injection with attenuated cultures, and Schultz has repeatedly produced a fibrinous pneumonia in rabbits by intravenous injections of attenuated pneumococci.

With the view of throwing more light upon this point, I undertook, with the aid of Dr. Rubin, a series of experiments upon six sets of rabbits (three in each set), of the same weight and inoculated with exactly equal doses of attenuated pneumococci, as follows:

Rabbit A in each set was inoculated intravenously, Rabbit B intratracheally, and Rabbit C also intratracheally, after having been previously etherized by the hypodermic injection of 1 c.c. of ether per pound of weight. With the hope of attenuating the pneumococci sufficiently so that the rabbits would not die of a rapidly fatal bacteriemia, and thus afford time for the development, perchance, of a pneumonia, the washed-off pneumococci of one blood-agar slant were suspended in 85 per cent. salt solution and placed in the incubator for twenty to twenty-four hours, as advised by Auskoff and Schultz. This was found insufficient, for most of the animals died within a very short period. The animals inoculated intratracheally after having been etherized invariably died first, and the leucocytosis was not nearly so marked. The ones inoculated intratracheally usually succumbed before the one receiving intravenous injections, both dying usually of a rapidly fatal pneumococcemia. This is interesting because it shows that the pneumococcus can penetrate into the circulation very rapidly from the respiratory tract, and may be considered a point in favor of the theory, advanced by Schultz and others, that the lung is the point of least resistance for the pneumococcus, and hence localizes there from a previous blood invasion.

Of the animals that survived long enough, there developed in two instances a pneumonia which resembled typical fibrinous pneumonia in man, and a bronchopneumonia in three others. In the former two it concerned intravenous, and in the rest, where the consolidation was not so sharply defined, intratracheal inoculations. In all of these there was a massive sero-fibrinous pleuritis, and in two a pericarditis. Pneumococci were isolated from the heart's blood in every instance. In a number of rabbits in this series, as well as in a large number of other rabbits which survived from twenty-four to forty-eight hours, or longer, the lungs frequently showed an active hyperemia in areas, which reminded one strongly of an early stage of red hepatization. To decide whether this condition was really an inflammatory process, and hence probably due to the pneumococcus, a number of specimens were placed in alcohol, sectioned, and stained for bacteria by Gram's method. In one of these I was able to demonstrate the presence of incapsulated diplococci within the alveoli, together with polymorphonuclear leucocytes, a large number of red blood corpuscles, and other cells. This rabbit was inoculated intravenously, and died twenty-eight hours after inoculation; it was examined within a few hours after death.

We have thus shown (1) that the pneumococcus when injected into the trachea in lethal doses is rapidly absorbed by the blood; (2) that when injected intravenously it may enter the alveoli of the lung within the short time of twenty-four hours; (3) that a fairly typical lobar pneumonia may develop after intravenous and intraperitoneal inoculation; (4) we have confirmed the view, expressed by Fraenkel, that pneumonia in the rabbit is more prone to occur after inoculation with attenuated pneumococci. Hence it does not seem altogether unlikely that the lobar pneumonia in man may be the result of a primary hematogenous infection.

III. PNEUMOCOCCEMIA IN RELATION TO THE LEUCOCYTOSIS AND THE CRISIS OF PNEUMONIA.

Accurate leucocyte counts were made simultaneously with the blood cultures in thirty cases. In four of these (see Table I), with a leucocytosis of 30,000 in two, and 13,800 and 12,340 respectively in the other two, the blood cultures were sterile. At first it would appear that this negative result might be due to a high leucocytosis, especially in the first two cases; yet from the fact that in the other two there was a low leucocytosis, and that in other cases positive results were obtained with equally high or even higher leucocyte counts, alike in fatal and non-fatal cases, it appears that there must be some other reason to explain these negative results of the blood cultures. Upon studying these cases more closely, it is found that in every instance except one the blood was obtained upon the day of crisis. Furthermore, in case No. 117, as the table will show, three cultures were made: one on the fifth day, with a negative result; one on the sixth day, with a positive finding; and again one on the seventh day, or the day of crisis, with a negative result. The leucocyte count remained practically the same (12,340). Again, in two of the fatal cases there was a marked reduction in leucocytes (1,600 and 1,040) shortly before death, whereas early in these cases there was a rather high leucocytosis (19,000 and 25,000). Clinically it has been determined, especially by the researches of von Jaksch, that prognosis in pneumonia is more serious when leucocytes diminish in number. That this is true there can be but little doubt. This leads one to raise the question whether there exists any causal connection between the disappearance of the leucocytes and the appearance of pneumococci in the circulating blood.

A number of observers, more particularly Williamson, have attempted to show, from a rather small number of observations in man, and mostly by analogy from experimental pneumococcus infection in the rabbit, that this diminution of leucocytes and the

TABLE I.

Relation of Pneumococcemia to Leucocytosis and to the Termination of Pneumonia.

No.	Age	Day of Disease	Days before D'th or Crisis	Leucocytes	Blood Culture	Termination
19......	27	7	0	6,000	+	Death
22......	27	6	4	30,000	+	"
23......	50	8	4	18,000	+	"
31......	33	10	10	22,000	+	"
73......	31	4	1	13,000	+	"
85......	30	6	2	17,450	+	"
87......	26	4	2	8,280	+	"
90......	56	2	1	1,600	+	"
92......	35	2	1	1,040	+	"
96......	22	6	3	16,000	+	"
99......	34	16	15	13,450	+	"
101......	36	4	4	12,000	+	"
35......	33	8	0	30,000	0	Recovery
49......	31	3	3	22,900	+	"
55......	19	8	3	30,000	0	"
77......	28	7	4	36,550	+	"
88......	15	6	2	17,250	+	"
89......	23	8	Lysis	25,000	+	"
93......	16	2	1	15,500	+	"
94......	40	5	2	12,500	+	"
95......	39	2	Lysis	10,640	+	"
98......	35	8	2	5,473	+	"
100......	40	7	2	17,350	+	"
102......	40	2	2	15,000	+	"
103......	36	5	2	14,250	+	"
108......	30	10	2	22,000	+	"
109......	40	7	2	23,600	+	"
112......	35	7	0	13,800	0	"
116......	40	5	3	19,450	+	"
117	36	5	2	11,256	0	"
....		6	1	12,340	+	
....		7	0	12,000	0	

total absence of leucocytosis in many fatal cases of croupous pneumonia are due to the "very special process" of the entrance of the pneumococci into the blood stream. Invasion of the blood by pneumococci occurs in nearly all fatal cases, and usually in large numbers, but this invasion is by no means to be regarded as an agonal event, because pneumococci have been found in the blood a number of times as early as the second or third day of the disease, and frequently as long as two, three, four, and a number of times six, days before death. Nor is blood invasion absent in the cases that recover, because it has not only been demonstrated in a large number of cases, by Prochaska, Fraenkel, Sittmann, Baduel, and in this series by myself, but the metastatic lesions in the most diverse organs observed every now and then in the course of even very mild cases of pneumonia show that the blood has contained the organism at least at some time during the infection. The practically constant pneumococcemia, even early in the disease in fatal cases, as well as in cases that ultimately get well, apparently independently of the number of leucocytes present, seems to indicate that pneumococcemia and leucocytosis in man are not necessarily so definitely associated, and that the diminution in leucocytes which does occur must be due to some other cause than the mere entrance of pneumococci into the blood stream. It may be more likely perhaps that the leucocytes are an index to the relative balance between the sum total of the infection, on the one hand, and the vital resisting agencies, on the other, and that hypoleucocytosis in pneumonia is an indication of failing resistance.

LEUCOCYTOSIS IN PNEUMOCOCCUS INFECTION OF THE RABBIT.

To test the conclusion drawn by Williamson that the diminution of the leucocytes in the pneumococcus infection of the rabbit is due to the penetration of the pneumococcus into the circulating blood, a series of experiments similar to his were instituted. After subcutaneous inoculation of lethal doses of highly virulent pneumococci, leucocyte counts simultaneously with blood cultures were made at frequent intervals, with these important additions: (1) Having previously shown that plain bouillon to which a small quantity of blood is added (7 loops per 5 c.c.) is a rather unfavorable medium for the pneumococcus, and having repeatedly been able to obtain growths from old cultures of pneumococci upon blood agar, but not upon glycerin agar, I made parallel cultures in ascites bouillon, blood agar, plain bouillon, and glycerin agar, using the same number of loops as did Williamson for each inoculation. (2) Believing also that a knowledge of the actual number of viable pneumococci in a given definite quantity of the animal's blood, together with a simultaneous count of the leucocytes, might throw some light upon the rôle these cells play in the infection, a series of blood-agar plates (5 to 7 drops of sterile blood to 5 c.c. of plain or glycerin agar) were also used.

A study of Table II will show that by the use of more favorable media for the growth of the pneumococcus, and incidentally by the use of a somewhat larger quantity of blood, the organisms have been cultivated much earlier in many instances than in the experiments of Williamson, Radziewski, and others, and than I was able to do upon the media they employed. Since the pneumococci are usually numerous upon the plates at the time, we are able to grow them in plain bouillon and glycerin agar; and because of the fact that their demonstration in the blood, often in considerable numbers, by this method of examination is possible long before the acme of the leucocytosis has been reached (from three to twenty-four hours in the rabbits with an early death, and to as long as seventy-two hours in the ones that lived a number of days), it may be concluded (1) that the hypoleucocytosis which develops in the course of a fatal pneumococcus infection of the rabbit is not due to the mere entrance of the pneumococci into the circulating blood, but rather perhaps to the exhaustion of protective agencies; and (2) that invasion of the blood in the rabbit, as in pneumonia in man, is to be regarded as a part of the disease-process, and not as a "very special" or ominous event. The latter conclusion is still further borne out by the fact that two rabbits in this series recovered, in one instance with as many as 12,000 pneumococci per loop of blood (the loop used in all these experiments contains approximately 1/1,000 c.c.).

Here I would also note the recovery of a number of other rabbits (not in this series), which were inoculated subcutaneously, after the presence of pneumococci had been demonstrated in the circulating blood.

That there exists a very close relation between the number of leucocytes and the number of pneumococci in the blood is evident, however, because in the fatal cases, up to a certain point, the rise in the number of leucocytes runs hand in hand with the increase in the number of pneumococci; again, after the leucocytosis has reached the acme, there is first a primary drop in both, just as if the disintegration of leucocytes causes a destruction of a number of the invading cocci (*vide* Table II, Nos. 1, 3, 5, 6, 8, and 10). This suggests that because of the exhaustion of the power on the part of the organism to produce new leucocytes, the pneumococci get the upper hand, and as the leucocytes diminish, the pneumococci increase in number. There is thus furnished by the blood-agar plate method a probable explanation of the observation, unaccounted for by Williamson, that at times his cultures remained sterile, while the earlier ones had shown pneumococci. This was associated in nearly every one of these instances with a rather sudden drop in the leucocytosis. Cultures upon blood agar would probably have shown pneumococci in the blood, though in diminished numbers, just as in my experiments.

In certain experiments in conjunction with Dr. Rubin it was found that alcoholized and etherized rabbits withstood pneumococcus infection much more poorly than normal rabbits; furthermore, that in the alcoholized and etherized rabbits the leucocytosis was not so marked, being at times wholly absent, while the number of pneumococci in the blood was greater than in the normal control rabbits.

These results seem to me to indicate that the leucocytes play an important rôle in the struggle against pneumococcus infection. This is in full accord with the fact that Kitasato and Wassermann, Buchner, and others have shown that *in vitro* the leucocytes have a definite bactericidal action (see Däubler, *loc. cit.*). The fall in the number of pneumococci shown by me to be coincident with the fall in the leucocytosis points directly to the liberation of pneumococcidal substances.

Filtrates of bouillon and serum cultures of pneumococci pro-

TABLE II.

Pneumococcemia and Leucocytosis in the Rabbit.

No.	Hours after Inoculation	Temperature (Rectum)	Leucocytes	BLOOD CULTURES						REMARKS
				Bouillon	Asites Bouillon	Gly. Agar	Blood Agar	Blood-Agar Plates		
								2 Loops	10 Loops	
1	½	101.8	11,340	0	0	0	+	0	10	Rabbit lived 1 mo. after inoc. At end of 3d week it developed pneumococ. arthritis of left knee joint; recovered. Death (finally) due to a massive pneumococ. pleuritis.
	4	102.4	12,840	0	..	0	+	15	0	
	12	103	15,250	0	+	0	+	25	144	
	24	100	18,840	+	+	0	0	30	165	
	48	102	8,440	0	..	0	+	7	30	
	54	104.8	3,520	+	..	0	+	15	100	
	66	101.8	10,520	0	..	0	..	7	40	
	114	101.6	8,680	+	+	+	+	116,640	260,560	
2	½	102.2	10,800	0	+	0	+	0	0	Death 37 hrs. after inoc.
	2	102	7,400	0	+	+	+	6,500	48,200	
	16	102.8	4,800	+	+	+	+	34,200	+	
	28	104.4	1,320	+	+	+	+	22,680	544	
	32	101.4	1,160	+	+	+	+	+	+	
3	2	102.8	10,450	+	+	0	+	11,059	118,317	Recovery.
	16	103	5,120	+	+	..	0	3,110	13,895	
	28	105	12,380	+	+	0	+	50	25,344	
	40	102.2	11,380	0	0	+	+	0	120,096	
	48	104	10,300	0	0	0	0	0	0	
	63	104.7	....	0	0	0	0	100	0	
	87	104.6	12,360	0	0	0	0	0	0	
4	2	103	11,450	0	+	0	+	17,560	150,000	Death 25 hrs. after inoc.
	8	105	14,336	+	+	+	..	40,360	+	
	16	103.3	6,000	+	+	+	+	16,430	+	
	24	101	1,100	+	+	+	+	+	+	
5	½	101	10,900	0	0	0	..	....	....	Death 92 hrs. after inoc.
	4	102	12,460	0	+	0	0	....	....	
	12	103	18,360	+	+	0	+	100	1,204	
	24	103.5	24,000	+	..	+	..	340	675	
	48	105	5,700	+	+	..	..	60	128	
	72	105	3,700	+	+	+	+	+	+	
6	½	100	12,000	0	0	0	0	....	....	Recovery.
	4	101	13,430	0	0	0	0	0	0	
	12	102	14,280	0	+	0	0	0	0	
	24	103.5	16,000	0	0	0	0	0	0	
	48	103.6	25,400	+*	0	0	0	0	1	*1 c.c. of blood was used to 5 c.c. of bouillon.
	72	103	27,000	0	0	0	+	75	....	
	92	104	25,300	0	0	0	0	0	0	
7	½	102	9,200	0	0	0	+	0	0	Death 85 hrs. after inoc.
	6	104.5	5,580	0	+	0	+	60	250	
	14	103.5	6,020	..	..	..	..	200	500	
	24	106.4	11,360	+	+	+	+	+	+	
	40	105	....	+	+	..	+	....	....	
	72	104	24,000	..	..	..	+	25	120	
	84	....	12,020	..	..	..	+	100,000	....	
8	½	....	9,600	0	0	0	+	0	0	Death 36 hrs. after inoc.
	3	103	21,560	+	+	+	+	188	1,778	
	6	103.8	13,120	0	0	0	+	84	166	
	12	104	12,450	+	+	0	+	45	237	
	24	104.5	11,100	0	+	+	+	100	1,442	
9	½	102.5	9,800	..	..	..	..	....	....	Death 49 hrs. after inoc.
	6	103.8	16,320	0	0	0	0	50	208	
	24	106.6	32,000	+	..	..	+	698	15,000	
	48	....	11,300	..	..	..	..	100,000	1,250,000	
10	½	102.4	9,845	0	+	0	0	0	0	Death 90 hrs. after inoc.
	6	104	14,000	0	+	0	+	0	0	
	24	105	38,700	+	+	+	+	8	74	
	48	103	14,120	0	0	0	+	2	14	
	72	98	12,600	+	+	+	+	1,234	5,678	
	86	99	8,870	0	+	0	+	6	34	

+ means very many.

duce in doses of 2–4 c.c. a slight rise of temperature and a considerable leucocytosis (18,000–28,000) in rabbits. Sterile bouillon and serum in the same quantities injected in the same way have no such action. This suggests that pneumococci may produce soluble toxins, and is in full accord with the observation that up to a certain point the increase in the number of leucocytes and in pneumococci go hand in hand in fatally infected rabbits.

IV. ACTION OF BLOOD AND SERUM ON PNEUMOCOCCI.

Media containing blood seems to be especially adapted for cultivating the pneumococcus. Washbourn advised the use of agar slants over which a few drops of rabbit's blood are allowed to flow as an excellent culture medium, and found that on it the pneumococcus lived much longer than is usual. Numerous parallel tests with different strains of pneumococci on rabbit's, human (both normal and pneumonic), dog's, and goat's blood-agar slants have been made in the course of this work. The efficiency of all these has been found the same. To determine further the constituent of the blood that especially favored the growth of the diplococcus, and thus to test the statement, made by Rymowitsch, that it was the hemoglobin, a hemoglobin infusion was made by laking washed corpuscles in distilled sterile water. The red fluid was floated over the surface of agar slants, protected from drying, and incubated for three days. Parallel tests were then made with the blood-agar slants and the hemoglobin medium; both media were found equally efficient, and both much more so than coagulated bovine serum, or glycerin agar, both as regards preserving vitality and securing abundance of growth.

In the course of certain experiments to determine the pneumocolytic effect of pneumococcus filtrates, it was shown that washed human and rabbit's corpuscles, when added to normal salt solution, increased materially the development and viability of pneumococci. The corpuscles at the same time were laked. By the use of blood-agar slants it has been found possible to preserve a moderate degree of virulence as long as 250 days, and in two instances a very high degree of virulence for 149 and 163 days. (In preparing blood agar plain agar preserves both vitality and virulence better than glycerin agar.) The tubes were kept in the incubator at 37 ° C., and drying was prevented by sealing with a sterile rubber cap the mouth of the tube after sterilizing in the flame. It is a well-known fact that the pneumococcus dies upon ordinary laboratory media in a very short time, and that even when transplanted frequently, and in this way kept viable, it loses its virulence very soon. The difference in the effect of ordinary glycerin agar and blood agar upon the pneumococcus is well brought out in the following experiments: A pneumococcus of standard virulence kept on blood agar was inoculated on the surfaces of three glycerin-agar and three blood-agar slants, which were placed in the incubator. At the end of twenty-four, forty-eight, and ninety-six hours, respectively, rabbits of the same weight were inoculated intraperitoneally. In each instance one rabbit was inoculated with pneumococci from the glycerin-agar and one with cocci from the blood-agar slant. The twenty-four-hour-old cultures produced death within twenty-four hours in both rabbits. After forty-eight and ninety-six hours of incubation, the two rabbits receiving the pneumococcus from the blood agar died promptly, while the other two, receiving the pneumococcus from the glycerin agar, survived. The forty-eight-hour-old culture produced some reaction, whereas the one incubated for ninety-six hours seemed not to affect the animal. Experiments like these lead one to believe that the conclusions drawn by numerous investigators upon the virulence or non-virulence of the pneumococcus, after having been grown on ordinary artificial media, are often erroneous and untrustworthy. Hence I would urge most emphatically, as Washbourn does, the use of blood agar, or other blood-containing media, in all investigations with the pneumococcus where, as is usual, a standard of virulence is indispensable for reliable results.

To determine further the influence of blood on pneumococci, parallel blood cultures were made in the following dilutions: 1:200, 1:100, and 1:8. Positive results were obtained in every instance of the 1:8 dilution, even when no pneumococci developed in the higher dilutions. This was constant with no exception. Now the chief difference between Prochaska's method, with uniformly positive results, and that of Cole was that the former used rather high concentrations of blood, and the latter rather high dilutions. The higher percentage of negative results in the early cases in this series, using higher dilutions than in the later cases, and the high total percentage of negative cases in Cole's series, I believe, can be attributed to this fact. Further, in a number of cases growths were obtained in pure pneumonic blood, when the cultures on bouillon and milk remained sterile. Case No. 116 showed this particularly well. The blood that was left after making the inoculations was defibrinated and placed in the ice-chest. Two weeks later a number of plates were made with blood agar, and there now developed in this medium an average of 350 colonies to the drop of blood. Whether there was any growth in the blood or not is difficult to say, yet it certainly shows that the blood drawn from a pneumonic patient possessed no bactericidal properties over the pneumococcus; and it also shows again that to conclude that pneumococci are absent in the blood because we fail to grow them on artificial media is incorrect. Further, by numerical determinations it has been shown that the pneumococcus multiplies rapidly when inoculated into fresh normal and pneumonic blood, so that blood in the medium, instead of having a bactericidal effect on the pneumococcus, distinctly favors its growth.

Has normal and pneumonic human serum any bactericidal action upon the pneumococcus? Behring found none; and the following reasons lead one to believe that such is the case: (1) Normal and pneumonic serum, especially when diluted with equal parts or more of bouillon, form a most excellent culture medium for the pneumococcus, growth being constantly more abundant than in bouillon only. The viability is increased if at the bottom of the tube blood corpuscles are present. (2) Living pneumococci in pneumonic serum survive for seventy-nine days when kept at 3 ° C. — a temperature to which the pneumococcus is otherwise very sensitive. (3) In Table III it is shown, by actual numerical determinations by the use of blood-agar plates, that normal human serum and blood and pneumonic serum appear to have no bactericidal power whatsoever over the pneumococcus.

The age of the sera in these tests varied up to twenty-four hours, and it was found that preserving the serum for twenty-four hours makes no difference. One-half c.c. of serum in each instance was inoculated with one loop of the water of condensation from blood-agar slants inoculated with the pneumococcus. After incubation (37 ° C.) for the different periods indicated in Table III, one loop was plated in blood agar and a careful count made of the number of colonies that developed. Blood-agar plates have the following advantages: (1) Owing to the small size and the

TABLE III.

Effect of Normal Human and Pneumonic Serum and Blood upon the Pneumococcus.

Serum	Pneumococcus	Immediately	3 Hours	5 Hours	24 Hours	48 Hours
Normal serum W	R 49	100	180	250	15,860	18,200
Normal serum W	116	48	260	480	1,000	4.500
Normal serum D	× 239	12,180	7,385	13,350	2,205	4,690
Normal serum D	42	11,180	20,520	22.400	∞	4,340
Normal serum D	116	200	510	7,700	∞	7,200
Normal serum D	69	4,480	8,400	∞	∞	.....
Normal serum S	1,386	1,004	1,824	3,684	40,000	.....
Normal serum S	R 46*b*	848	1,368	1,824	10,456	.....
Normal serum S	46	4,856	18,414	55,242	∞	.....
Normal serum H	1,386	840	2,268	17,995	∞	.....
Normal serum H	R 46*b*	912	1,386	7,824	∞	.....
Normal serum H	47	16,800	29,127	∞	∞	.....
Normal serum A	1,386	2,144	9,800	14,120	∞	.....
Normal blood D	41	50	432	168	12,600	.....
Normal blood S	R 46*b*	4.848	18,414	55,414	∞	.....
Normal blood H	R 46*b*	6,912	32,352	∞	∞	.....
Pneu. serum 116*	1,386	1,440	5,720	6,590	∞	.....
Pneu. serum 116	41	108	5,720	10,450	∞	.....
Pneu. serum 117†	R 49	1,360	1,540	1,470	∞	4,200
Pneu. serum 117	× 239	3,570	7,385	9,860	14,000	7,280
Pneu. serum 117	42	11,040	15,000	∞	11,340	5,390
Pneu. serum 117‡	R 49	140	280	∞	∞	4,270
Pneu. serum 117	116	66	33	78	280	13,700
Pneu. serum 117	× 239	12,600	16,280	21,160	5,775	6,930
Pneu. serum 117	69	7,280	7,560	∞	∞	.....
Rabbit's blood 43	46*b*	6,200	14,722	∞	∞	.....
Rabbit's serum 43	42	7,392	∞	∞	.....	.....
Controls milk	1,386	8,000	10,880	∞	∞	.....
Controls milk	41	41	100	776	∞	.....

* Blood was drawn on the day before crisis with a leucocytosis of 17,450.

† Blood was drawn on the day before crisis with a leucocytosis of 12,460.

‡ Blood was drawn on the day of crisis with a leucocytosis of 12,000.

∞ Stands for innumerable.

transparency of the colonies of the pneumococcus, their recognition on plain or glycerin-agar plates is made very difficult; this is removed to a large degree because upon this medium the colonies not only grow somewhat larger, but have a decided greenish color. (2) Careful controls with glycerin agar show that upon the blood medium there constantly develop a larger number of pneumococci, and thus by its use we obtain a more accurate knowledge of the number of viable cells present. (3) This medium affords the opportunity at the same time to study the hemolytic effect of the pneumococcus, and it has been found that highly virulent pneumococci (such as have been passed through a series of rabbits) possess a greater power of hemolysis than do those of a lower degree of virulence, and that the pneumococcus when grown in the serum, both natural and pneumonic, soon loses its hemolytic power. The diminution in the number of colonies which occurs after forty-eight hours' incubation is clearly due to exhaustion, and not to a bactericidal effect; for, where the number of micro-organisms was small in the beginning, and hence the development rather more slow, this diminution is not noticed, but a continued multiplication instead.

The pneumonic sera were carefully controlled in every instance with normal sera from different individuals. It has been found that, while there is no difference in the rapidity with which development of pneumococci occurs, nor with the rapidity of the death of the cocci later, there is this difference that there results early a marked turbidity, and later an abundant precipitate, together with agglutination, in the pneumonic serum, whereas the normal serum remains practically clear, containing, however, equally many cells. This marked turbidity and precipitation in pneumonic serum has led to the erroneous conclusion, by a number of investigators, that it was due to an enormously rapid multiplication of pneumococci.

V. AGGLUTINATION OF THE PNEUMOCOCCUS.

The pneumococcus when cultivated in the sera, as well as when injected into the body, of susceptible animals, appears in typical diplococcus forms, and occasionally in very short chains.

In the sera of less susceptible animals—*i. e.*, the dog, cat, or horse—Kruse and Pansini noted the tendency of the organism to grow in chains and bunches. Wadsworth, however, very recently states that he has been unable to confirm their findings. Metchnikoff first observed the tendency toward chain formation in the serum of immunized rabbits. Later Arkharow, Issaef, Mosney, Washbourn, and others noted that this tendency to the formation of chains and bunches in the serum of immunized animals was associated with what seemed to be definite evidences of degeneration of the organisms, and believed it to be closely related to the degree of immunization. Bensançon and Griffon considered the growth in clumps and chains in the sera of specially immunized animals so marked and characteristic that they described it as agglutination; they were the first observers to apply this reaction to human sera from croupous pneumonia and other pneumococcus infections. Their method consisted in inoculating 1–2 c.c. of undiluted serum with pneumococci, and observing the growth at the end of twenty-four hours. If the reaction was positive, the bottom of the test-tube would show a membrane or a gelatinous mass composed of clumps and chains of pneumococci. They were rarely able to obtain agglutination before the day of crisis, and they found the agglutinating substance to diminish rapidly thereafter.

Neufeld later studied the agglutinating action of the serum of immunized animals on pneumococcus-bouillon cultures. He used various dilutions up to 1 : 50, and was the first observer to note agglutination of pneumococci microscopically and macroscopically by the usual method. He emphasized the importance of using fresh serum, and found that agglutination took place in from one-fourth to one-half hour. Pneumococcus agglutination was studied by Huber, Daddi, and Pesci, and recently by Wadsworth, who has devised a new technic, which he believes is more reliable and delicate than the methods hitherto employed. In this work the reaction of blood serum from cases of croupous pneumonia or other pneumococcus infection was exclusively studied. For the controls, which were carefully made in every instance, normal human serum was used. Both the method of Bensançon and

Griffon, but using various dilutions of serum (as high as 1:50), and that of Neufeld were employed. And while the latter is no more delicate, it has the advantage that (1) results can be obtained in a shorter period; the process being complete in from one-half to several hours; (2) the microscopical changes in the process can be more easily watched; and (3) it eliminates practically the rapid growth of organisms which occasionally seems to occur, especially in the sera in which the complement has been destroyed, either by heat or age. It has, however, the decided disadvantage that it is often difficult to get a concentrated bouillon culture of pneumococci.

After twelve to twenty-four hours' incubation in the former method, and earlier in the latter, the liquid becomes perfectly clear, and the growth forms a membrane or a gelatinous mass at the bottom of the tube, which does not readily mix with the clear serum upon shaking, especially if the agglutination is marked. When the reaction is more feeble, and in the higher dilutions, a whitish, flocculent precipitate is thrown down. This readily mixes with the overlying liquid, which is usually more or less turbid. The sediment in each instance is early composed of swollen diplococci, single, in long chains or bunches, and late of a highly refractive, nonstaining, granular substance.

When equal quantities of agglutinating pneumonic serum and pneumococcus bouillon culture are placed in the hanging drop, the single cocci swell to twice and three times their normal size; the opposing ends become flattened, their outline indistinct, and the cells homogeneous. In the center of each coccus is seen a very small, round, highly refractive spot. Long chains and clumps are often seen. Ultimately they lose their affinity for the ordinary stains, as Neufeld has shown. These changes were further studied by the use of the capsular stains recommended by Welch, Hiss, and Boni. Frequently empty capsules and what appeared as degenerated fragments of cells in capsules, in clumps or single, were seen. Capsules of enormous size are often demonstrable early in the process. This entire picture gives the impression of a rapid lysis or degeneration. In short, the changes observed appear to be identical with those described, first by

Radziewsky in the subcutaneous tissue of the rabbit, and later by Neufeld in his study of the Pfeiffer phenomenon with the pneumococcus. However much this process appears as a rapid destruction and death of the cells, the following reasons would lead one to believe that this is only apparent and not real:

1. Neufeld has shown that dead pneumococci agglutinate equally as well as the living organisms, and that by simply heating almost to boiling the cocci forming the agglutinated masses in which no organisms can be distinguished again take on normal shape and outline. Their affinity for the ordinary stains also returns.

2. I was able repeatedly to grow the organisms from the masses agglutinated most perfectly, in which no organisms could be demonstrated in stained preparations, almost as well as from the controls in which organisms were abundant. Living organisms were demonstrated as long as twenty-five days after the agglutination tests were made. Further, agglutinated and supposedly dead pneumococci were found to be virulent to rabbits in a number of cases, the pneumococcus being isolated in pure culture from the rabbit's blood after death in each instance.

3. Further, by the use of the plate method with blood agar, I have been able to show that the development of the organisms and their death coincide very closely indeed when grown in normal serum, which remains practically clear, and when grown in pneumonic serum, in which there is an early agglutination and later an abundant sediment. Hence the conclusion drawn by a number of investigators, based upon the microscopic picture, that this granular substance is the result entirely of a rapid lysis of pneumococcus cells, is probably incorrect. (See section on the production of acid by the growth of pneumococci in pneumonic serum.)

Results.—In the total number of 200 tests the serum from 96 cases of pneumonia and 55 strains of pneumococci were used. In the main, the results of the previous investigators have been confirmed. The serum on the second day in one case showed distinct agglutinative properties, agglutinating its own and, more feebly, other pneumococci. Agglutination grows more marked as crisis is reached, and diminishes gradually thereafter. The serum of one case showed rather marked agglutinative power three weeks after crisis. This case later developed empyema, which was found to be due to the pneumococcus.

Aging the serum, both on ice and at room temperatures, diminishes its powers of agglutination, but my results do not show the rapid diminution spoken of by Neufeld. Marked agglutinating properties were found several times as late as thirty days after the serum was drawn. Bensançon and Griffon have found that occasionally pneumonic serum agglutinated only pneumococci derived from the same patient. While pneumococci and serum from the same patient agglutinate most perfectly, yet in no instance did I fail to obtain agglutination with pneumococci of different strain. The highest dilutions at which agglutinations could be obtained were 1:40 or 1:50. The agglutinin seems to be as abundant in the serum obtained by allowing the blood to clot as in that obtained by immediate defibrination and centrifugalization.

After a careful analysis of my results, I am unable to establish any constant difference in the degree of agglutination in the sera of the fatal and non-fatal cases.

Owing to the almost uniformly positive results of the blood cultures, I am unable to state definitely whether or not there exists any constant relation between the presence of the organisms in the blood and the abundance of the agglutinin. Nor has it been possible to establish any definite relation between the abundance of the agglutinin and the height of the leucocytosis. In a number of instances where there was a high leucocyte count the serum possessed strong agglutinating properties. However, the sera in three of the cases (Nos. 55, 35, and 24), in which the organisms failed to grow in cultures, showed definite agglutination. Yet in smears from the blood the organisms were demonstrable. The serum from two cases of epidemic cerebrospinal meningitis had no agglutinative power over pneumococci, but did agglutinate the diplococcus intracellularis. Erysipelas serum was found to agglutinate the pneumococcus in dilution below 1:20 only.

VI. THE PRODUCTION OF ACID BY THE GROWTH OF PNEUMOCOCCI IN PNEUMONIC SERUM.

Pneumococci grow equally well in normal and pneumonic serum (Table III). In the former it produces scarcely any appreciable precipitate, while in the latter a voluminous sediment appears, often comprising at the end of forty-eight hours one-half of the serum in the test-tube. This precipitation is a distinct reaction, and has apparently nothing to do with the agglutination which occurs very early. That this voluminous sediment is the result of an exceedingly rapid growth and death of the pneumococci, as the picture under the microscope suggests, and as Neufeld, Wadsworth, and others would lead us to believe, seems unlikely, because by the plate method I have shown that the development and death of the cells in this serum follow the same course exactly, as in normal serum. Furthermore, both Neufeld and Wadsworth obtained similar precipitates by adding serum of animals immunized against pneumococci to culture fluids and to pneumococcal suspensions in salt solution from both of which the cocci had been removed.

In trying to determine to what the precipitation so constantly observed in pneumonic sera might be due, the reactions of the sera were taken at intervals after inoculation with the pneumococcus, and it was found that the growth of the pneumococcus in pneumonic serum results in the production of a marked acid reaction (Table IV), while in normal serum the reaction remains alkaline. This has proved to be constant. The reaction as a routine was tested by means of a platinum loop and a good quality of litmus paper. Accurate titrations have been made often enough to prove the efficiency of litmus paper as an indicator. The acidity ranged from 1 to 2.3 per cent. to litmus, and as high as 3 per cent. to phenolphthalein. The acidity develops rapidly and in direct proportion to the rapidity of growth. It is usually quite marked after eighteen hours' incubation, increasing until living micro-organisms are no longer demonstrable; after this time the reaction does not change. The pneumonic sera tested in this way were obtained in all stages of the disease from the third day to one day after crisis. No attempt has been made to isolate the acids other than to determine that lactic acid is present. Filtering pneumonic serum through porcelain filters does not remove the substance or substances upon which the acid production depends, while dialysis does. By growing the pneumococcus in dialyzed pneumonia serum, the reaction remains unchanged. Keeping the serum in the ice-chest for as long as forty-three days does not change the property of acid production when pneumococci are inoculated. Heating pneumonic serum to 56° and 60° for one-half hour delayed and diminished the acid production in all of fifteen sera so tested, while in five no acid appeared. The presence of red blood corpuscles in the serum does not seem to modify the production of acid.

That the precipitate constantly observed when pneumococci grow in pneumonic sera is due to the production of acids is probably true, for the following reasons:

1. Precipitation is proportional to the degree of acidity present.
2. It is absent in heated serum when the reaction remains alkaline.

TABLE IV.

The Production of Acid by Growth of Pneumococcus in Pneumonic Serum.

NATURE OF SERUM	NO. OF SERA TESTED	REACTION											
		18 Hrs.			48 Hrs.			60 Hrs.			84 Hrs.		
		Alk	N.	Ac.	Alk	N.	Ac.	Alk	N.	Ac.	Alk	N.	Ac.
Pneumonia	32	3	1	28	..	1	31	..	..	32	..	..	32
Normal	28	28	..	..	28	..	..	28	..	..	28	..	..
Pneumonic—heated to 60° C. for 30 minutes	6	4	..	2	3	..	3	2	..	4	2	..	4
Pneumonic—heated to 56° C. for 30 minutes	9	7	2	..	3	..	6	3	..	6	3	..	6
Pneumonic—dialyzed	4	..	4	..	..	4	..	..	4	..	..	4	..
Pneumonic—filtered	1	1	..	..	.	..	1	..	..	1	..	..	1
Scarlet fever	5	..	..	5	..	..	5	..	..	5	..	..	5
Scarlet fever—heated to 56° C. for 30 minutes	4	4	..	..	4	..	4	4	..	..	4	..	..

NOTE.—As a rule, 0.5 c.c. of serum was inoculated with pneumococci. Blood-agar plate cultures were made with fixed quantities immediately, after eighteen hours, and after forty-eight hours, and the colonies counted. The number of colonies increased and decreased in all sera in the same manner as is shown in Table III. The pneumococci did not die out more rapidly as the acid reaction appeared in pneumonic and scarlet fever serum than in the normal and the heated sera.

3. A similar precipitate is produced by the addition of an organic acid to the culture in normal serum and in heated pneumonic serum.

4. By restoring the original alkalinity of the mixture a considerable amount of the precipitate is dissolved.

I have found also that pneumococci produce acids in the serum of patients in other febrile diseases than pneumonia, especially in scarlet fever. Here it also produces a sediment similar to that obtained in pneumonic serum, but not so abundant. Here too the pneumococcus may be agglutinated.

Numerous experiments have been made to test the reaction of normal human serum in which pneumococci were grown. A large number of different strains of pneumococci were used. In every case the reaction which was tested at the end of eighteen, twenty-eight, sixty, and eighty-four hours after inoculation remained alkaline. In order to determine definitely that actual growth took place, colonies were counted immediately, at the end of eighteen, and after forty-eight hours, the results in this respect corresponding closely to those given already in the tables.

Streptococci do not produce any change in reaction when grown in pneumonic serum. The same is also true of staphylococcus aureus.

The production of acids by the growth in the test-tube of pneumococcus in the serum of pneumonia patients naturally leads one to raise the question whether this does not also occur in the consolidated lung and in the blood of patients. This is not improbable in view of the facts that when the pneumococcus is grown in extracts of consolidated lungs it produces acids, and that late in fatal cases of pneumonia there is a diminished alkalinity of the blood (von Jacksch). Perhaps some of the toxic symptoms in pneumonia are due to acid intoxication. If this is so, it would explain the good results reported by some from the administration of alkalinizing agents. Unfortunately I have not had the opportunity to study carefully the time of appearance and disappearance of the acid producing power of pneumonic serum, and important questions such as its possible relations to crisis, must be left for future consideration.

VII. HEMOLYSIS IN FATAL PNEUMOCOCCUS INFECTIONS OF RABBITS.

In the course of my experiments attention became directed to the relatively early hemolysis of the blood invariably present in rabbits dead from a rapidly fatal dose of the pneumococcus. Other rabbits dead from other causes than infection, such as traumatism, hemorrhage, chloroform, and ether anesthesia, did not show this. The blood was carefully drawn into small pipettes, the end sealed, and immediately centrifugated to separate the corpuscles from the serum. To be able to express in percentages the degree of hemolysis present, an arbitrary colorimetric scale of solutions of carmine in sealed pipettes of the same diameter was constructed. That post-mortem hemolysis in general is mostly due to the growth of micro-organisms seems likely, because in animals dead from traumatism there is practically no solution of the corpuscles as long as the blood is sterile, but as soon as the blood becomes invaded by bacteria, hemolysis rapidly increases. That the hemolysis of the blood in pneumococcus infection of rabbits is probably due, wholly or in part, to the action of pneumococci seems likely for various reasons. In a number of animals there was a slight hemolysis shortly before death, while the blood was crowded with pneumococci. The pneumococcus, especially when highly virulent, produces an appreciable zone of hemolysis (which is always greenish in color) around the colonies on blood agar. (It may be of interest to note here that the surface colonies produce a greater degree of hemolysis than do the deep colonies; indeed, it is frequently absent in the latter, just as if oxygen were necessary to produce this phenomenon.)

When rabbit's blood is inoculated with pneumococci in the test-tube, the increase of hemolysis seems to run hand in hand with the increase of the pneumococci. This is shown by the plate method in the following experiment, repeated a number of times: Three test-tubes, each containing 1 c.c. of rabbit's blood, were taken, one used as a control, and the other two inoculated with a large number of highly virulent pneumococci. One of these, with the control, was placed in the incubator, and the other one in the ice-chest. At the end of twenty-four hours the tube in the incubator containing the pneumococcus showed a high degree of hemolysis and multiplication of pneumococci, while the one in the ice-chest, where the number of pneumococci remained stationary, showed no hemolysis. The heat of the incubator alone seems to produce only a slight hemolysis, as shown in the control. Again, similar experiments with the blood of rabbits, dead from the pneumococcus infection, and crowded with pneumococci, show that the hemolysis increases rapidly in the incubator, while the pneumococci rapidly decrease in number, just as if their disintegration liberated a hemolytic substance. Where the blood is put in the ice-chest, the hemolysis, instead of remaining the same as in the former instance, shows some increase. In some cases, as the hemolysis increased, the plates showed a diminution of the number of cocci.

These observations led me to test the hemolytic properties of various filtrates of a highly virulent pneumococcus. Filtrates obtained from bouillon and milk cultures incubated for from twenty-four hours to several days, and from cultures in heated (56° for thirty minutes) and unheated human serum, and in equal parts of heated human and rabbit's serum, showed no hemolysis.

The filtrates of infected rabbit's blood, incubated for twenty-four hours, caused distinct hemolysis of fresh washed rabbit corpuscles in two instances, and a trace in the third. This power was destroyed by heating to 56° C. for thirty minutes.

VIII. DIFFERENTIATION OF PNEUMOCOCCI AND STREPTOCOCCI.

Quite independently of Schottmüller's recent work, I have found that in the use of blood-agar plates we have a seemingly reliable and simple method of differentiating pneumococci from streptococci. The blood-agar plates were prepared by mixing 0.3–0.5 c.c. of sterile defibrinated blood (rabbit or human) to the tube containing 5 c.c of melted plain agar cooled to 45° C.; it is best not to get more than 300 colonies per plate. Upon this medium the colonies of pneumococci grow larger, are themselves of a distinct green color, and are surrounded by a narrow zone in which the corpuscles are destroyed. This zone is never clear and transparent, but always opaque and of a distinct greenish tinge. The colonies of streptococci, on the other hand, remain small and grayish, and are surrounded by a perfectly clear transparent zone of hemolysis, the size of which is in direct proportion to their virulence. Sixty-five strains of pneumococci have been tested. These were obtained mostly from the blood; a number of them came from the consolidated lung and from the sputum of pneumonia patients. The results outlined above were constant without exception.

A pneumococcus whose virulence had been increased to such a degree that the intravenous inoculation of 1/1,000 of a loop of broth culture caused death in a rabbit in twenty-four hours produced a wider zone of hemolysis, if we may call it so, than did the organisms of less virulence, but even here the zone was not clear and transparent.

Thirty-five strains of streptococci have been tested. For most of these I am indebted to Dr. Weaver and Dr. Ruediger, and in every instance the colonies remained small and surrounded by a well-marked clear zone of hemolysis. My results are therefore in full accord with those previously obtained by Schottmüller.

The fact that pneumococci produce acid reaction in pneumonic serum, whereas streptococci do not, so far as I have observed, may be mentioned as another point of difference between these organisms.

IX. CONCLUSIONS.

The following conclusions are believed to be justifiable:

1. With improved technic, using for inoculation large quantities of blood, the pneumococcus can be recovered in practically all cases of croupous pneumonia, and in obscure cases of pneumococcus infection blood cultures may be a diagnostic method of positive value.

2. Pneumococcemia in pneumonia does not mean an especially unfavorable prognosis, and is to be regarded here, as in subcutaneous pneumococcus infections of the rabbit, not as an especially ominous or agonal process, but rather as an integral part of the infection.

3. There seems to be a diminution, either in the number or viability, or both, of the pneumococci in the blood at the time of crisis.

4. The number of leucocytes in pneumonia in man and in the pneumococcus infection of the rabbit is an index to the degree of resistance, and the leucocytes probably constitute an important factor in combating the infection. The clinical observation that a high leucocytosis means a favorable prognosis irrespective of the presence of pneumococci in the blood is undoubtedly correct.

5. The hypoleucocytosis developing upon a previous hyperleucocytosis during the course of many fatal cases of lobar pneumonia (and in pneumococcus infection of the rabbit) cannot be looked upon as due to the entrance of the pneumococcus cells into the blood stream, but probably rather as the result of an exhaustion of the resisting powers.

6. The leucocytosis may be incited, at least in part, by soluble substances liberated by pneumococci.

7. It was not possible to establish any appreciable difference in the degree of virulence of the pneumococcus isolated early or late in the course of the disease, nor in the fatal or non-fatal cases.

8. Fresh normal and pneumonic blood and serum have no bactericidal influence upon the pneumococcus. Whatever other differences they may have, so far as this point goes the serum from pneumonia patients behaves exactly as does normal serum.

9. The interesting question whether lobar pneumonia is the primary result of a direct local infection of the lung, or a secondary localization of a primary blood invasion, is as yet hardly ripe for final discussion, but that the latter does occur, at least in some instances, is not altogether unlikely.

10. Agglutination of the pneumococcus by pneumonic serum is constant, but the conclusion drawn by certain investigators that the voluminous sediment and the early clouding of certain immune sera in which the pneumococcus is cultivated are due to a rapid lysis of the cocci seems incorrect. It seems to concern a precipitate the result of acids which appear when pneumococci are grown in pneumonic serum.

11. The production of acids by pneumococci in pneumonic serum suggests that some of the toxic symptoms of pneumonia may be due to acid intoxication. This needs further study.

12. Since the viability and the virulence of the pneumococcus are preserved for a remarkably long period upon blood agar, we have in this medium an important aid to all investigations of this micro-organism. The constituent of the blood which has this rather remarkable effect is probably the hemoglobin. Blood-agar plates are valuable for differentiation of pneumococci and streptococci.

13. That the pneumococcus by its growth in rabbits produces a soluble hemolysin for the corpuscles of this animal is probably true. That this is either small in amount or unstable, or both, is likely.

LITERATURE.

BADUEL. *Riforma med.*, 1899, 11, p. 170.
BANTI. *Sperimentale*, 1890, 14, p. 349.
BELFONTI. *Riforma med.*, 1890, 6, p. 769.
BENSANÇON AND GRIFFON. *Ann. de l'Inst. Pasteur*, 1900, 16, p. 449.
BERGHINI. *Clin. med. ital.*, 1899.
BESREDKA. *Ann. de l'Inst. Pasteur*, 1901, 15, p. 880.
COLE. *Bull. Johns Hopkins Hosp.*, 1902, 13, p. 136.
DADDI E PESCI (Florence). *Riv. crit. de clin. med.* 1901, 21. Abstr., *Centralbl. f. inn. Med.* 1901, 38, p. 938.
DÄUBLER. *Centralbl. f. Bakt.*, 1899, 25, p. 129.
FRAENKEL. *Deutsche med. Wchnschr.*, 1901, 27, p. 298.
HUBER. *Centralbl. f. inn. Med.*, 1902, 23, p. 417.
JACOB. *Ztschr. f. klin. Med.*, 1898, 30, p. 5.
v. JAKSCH. *Centralbl. f. klin. Med.*, 1892, 23, p. 187.
KLEMPERER. *Berl. klin. Wchnschr.*, 1891, 28, 833, p. 869.
KOHN. *Deutsche med. Wchnschr.*, 1897, 23, p. 186.
KRUSE UND PANSISI. *Ztschr. f. Hyg.*, 1892, 11, p. 279.
KÜHNAU. *Ztschr. f. Hyg.*, 1894, 25, p. 492.
LIBMAN. *Jour. of Med. Resch.*, 1901, 1, p. 84.
NAZARI. *Riforma med.*, 1897, 13, p. 243.
NEUFELD. *Ztschr. f. Hyg.*, 1902, 40, p. 54.
ORTHENBERGER. *Münch. med. Wchnschr.*, 1888, 35, pp. 853, 873.
PANE. *Riforma med.*, 1899, 15, p. 174.
PÄSSLER. *Münch. med. Wchnschr.*, 1901, 48, p. 289.
PIERACCINI. *Accad. med. phisica florentina*, May 3, 1899.
PROCHASKA. *Centralbl. f. inn. Med.*, 1900, 21, 1145. *Deutsch. Arch. f. klin. Med.*, 1901, 70, p. 559.
RADZIEWSKY, *Ztschr. f. Hyg.*, 1901, 37, p. 24.
RUEDIGER. *Jour. Am. Med. Ass.*, 1903, 41, p. 962.
RYMOWITSCH. *Centralbl. f. Bakt.*, 1902, 32, p. 385.

SCHOTTMÜLLER. *Münch. med. Wchnschr.*, 1903, 50, pp. 849, 909.

SCHULTZ. *N. arch. des sciences biol. d. St. Petersburg*, 1901, 8, p. 1.

SELLO. *Ztschr. f. klin. Med.*, 1898, 36, p. 112.

SILVESTRINI AND SERTOLI. *Riforma med.*, 1899, 15. Abstr., *Centralbl. f. inn. Med.*, 1900, 21, p. 135.

SITTMANN. *Deutsch. Arch. f. klin. Med.*, 1894, 53, p. 323.

WADSWORTH. *Jour. of Med. Resch.*, 1903, 10, p. 228.

WASHBOURN. "Croonian Lectures," *Lancet*, London, 1902, 163, p. 1301.

WEAVER. *Jour. Infect. Dis.*, 1904, 1, pp. 91, 106.

WELCH. *Johns Hopkins Hosp. Rpts.*, 1892, 3, p. 125.

WHITE. *Jour. Exp. Med.*, 1899, 4, p. 425.

WILLIAMSON. *Ziegler's Beiträge*, 1901, 29, p. 41.